

Supplementary materials:

Materials:

Materials. Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich, all restriction enzymes were obtained from New England BioLabs (NEB) and all cell culture products were purchased from GIBOC (Gibco BRL/Life Technologies, a division of Invitrogen.).

siRNAs. siRNA and antisense strand RNA were purchased from Integrated DNA Technologies (IDT).

Anti-*tat/rev* 27 mer siRNA: Sense sequence: 5'-
GC GGAGACAGCGACGAAGAGCUCAUCA-3';

Antisense: 5'- UGAUGAGCUCUUCGU CGCUGUCUCCGCdTdT-3';

Anti-*tat/rev* 21 mer siRNA: Sense sequence: 5'-
GC GGAGACAGCGACGAAGAGC-3';

Antisense: 5'- GCUCUUUCGU CGCUGUCUCCGCdTdT-3';

Aptamer-siRNA chimeras. (The 27 or 21 mer sense strand is marked in bold, the linker (*CUCU*) is indicated in italics and mutated nucleotides are underlined).

Aptamer:

5'- GGGAGACAAGACUAGACGCUCAAUGUGGGCCACGCCCGAUUU
ACGCUUUUACCCGCACGCGAUUGGUUUGUUUCC - 3'

Ch L-1 sense strand:

5'- GGGAGACAAGACUAGACGCUCAAUGUGGGCCACGCCCGAUUU
ACGCUUUUACCCGCACGCGAUUGGUUUGUUUCCCC*CUCU***GCGGAGACAGC**
GACGAAGAGCUCAUCA -3'

Ch 1 sense strand:

5'- GGGAGACAAGACUAGACGCUCAAUGUGGGCCACGCCCGAUUU

ACGCUUUUACCCGCACGCGAUUGGUUUGUUUUCCGCGGGAGACAGCGACG

AAGAGCUCAUCA -3'

Ch L-2 sense strand:

5'- GGGAGACAAGACUAGACGCUAAUGUGGGCCACGCCGAUUUU

ACGCUUUUACCCGCACGCGAUUGGUUUGUUUUCCC CUC UGC GGAGACAGC

GACGAAGAGC -3'

Ch 2 sense strand:

5'- GGGAGACAAGACUAGACGCUAAUGUGGGCCACGCCGAUUUU

ACGCUUUUACCCGCACGCGAUUGGUUUGUUUUCCC CUC UGC GGAGACAGC

AAGAGC -3'

M-1 sense strand:

5'- GGGAGACAAGACUAGACGCUAAUGUGGGCGGGGCCGAUUUU

ACCGUUUUCAAAGCACGCGAUUGGUUUGUUUUCCC CUC UGC GGAGACAGC

GACGAAGAGCUCUCAUCA -3'

M-2 sense strand:

5'- GGGAGACAAGACUAGACGCUAAUGUGGGCCACGCCGAUUUU

ACGCUUUUACCCGCACGCGAUUGGUUUGUUUUCCC CUC UGC GGAGACAGC

GUGUAAGAGCUCUCAUCA -3'

Ch L-1, Ch1 and M-1 antisense strand:

5'- UGAUGAGCUCUUCGUCGCUGUCUCCGCDdT-3'

Ch L-2, Ch 2 antisense strand:

5'- GCUCUUCGUCGCUGUCUCCGCDdT-3'

M-2 antisense strand:

5'- UGAUGAGCUCUUACACGCUGUCUCCGCDdT-3'

Figure Legends.

Fig S1: Gene silencing activity and strand selectivity of chimeras RNAs and siRNA. Dual luciferase assays of psiCHECK sense (white bars) and anti-sense (gray bars) targets are shown. All RNAs are normalized to the value of the corresponding buffer control. The strand selectivity was calculated: $R_{\text{buffer}} = 1.0$; $R_{\text{27 mer siRNA}} = 2.2$; $R_{\text{21 mer siRNA}} = 4.9$; $R_{\text{Ch L-1}} = 3.2$; $R_{\text{Ch L-2}} = 1.9$; $R_{\text{Ch 1}} = 2.9$; $R_{\text{Ch 2}} = 1.6$; $R_{\text{M-2}} = 1.2$, respectively.

Fig S2: Images were combined and deconvoluted to reconstruct a three-dimensional image. Three-dimensional image reconstruction shows localization of the Cy3-labeled **Ch 1** in a single cell.

Fig S3: RACE PCR sequence.

Figure S4: Immunofluorescence assay of HIV-1 p17. HIV-1 infected CEM cells were incubated with 400 nM of aptamer or chimeras (**Ch L-1** and **Ch L-2**) in culture medium for **a**) 24 hours and **b**) 72 hours. Cells were washed with PBS, fixed, permeabilize and block with NGtS. After incubation with primary antibody (anti-p17), FITC-conjugated secondary antibody (H_o-α-Mu-FITC) was added to stain cells. Cells were washed, resuspended in 15 µL hard mounting medium and spot on a microscopy slide for confocal microscopy.

Figure S5: IFN assays. IFN-β, the interferon response gene encoding P56 (CDKL2) and OAS1, mRNAs were measured by quantitative RT-PCR. The expression of these interferon response genes was, not significantly induced by the siRNAs or chimeric RNAs, whereas expression of these genes was induced by poly(IC) in HEK 293 cells **a**) or by IFN-alpha in infected CEM cells **b**). Gene expression levels are normalized to GAPDH mRNA expression levels. The data represent the average of triplicate measurements.

Figure S1:

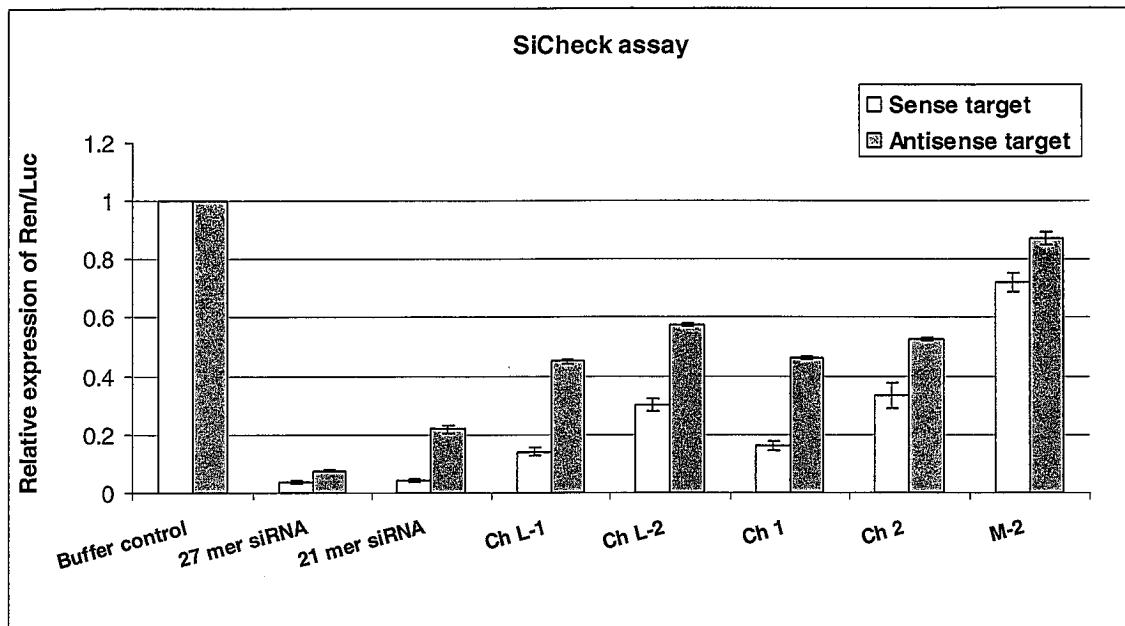


Figure S2:

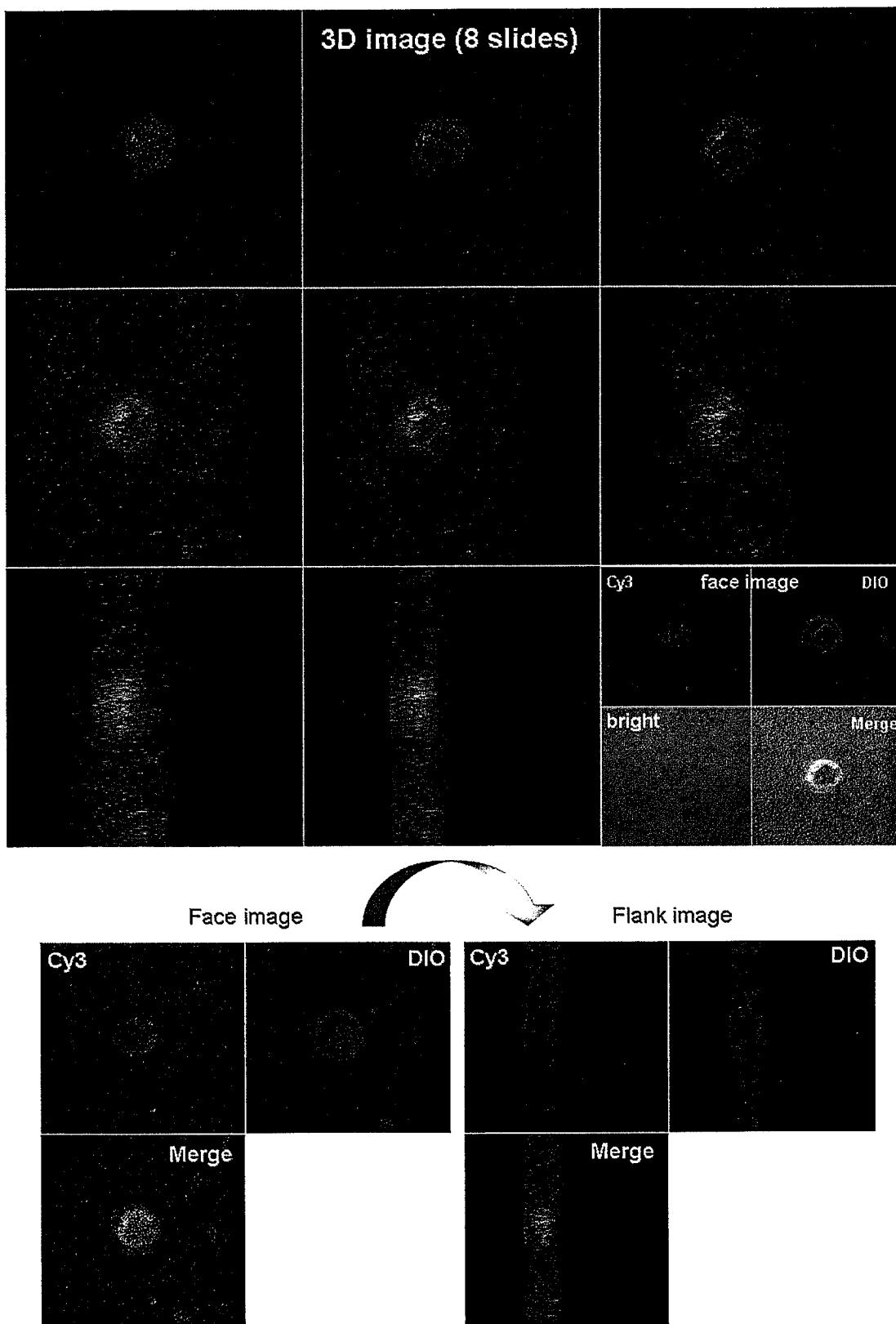


Fig S3:

For 27 mer duplex RNA, RACE PCR product was cloned into TA vector and sequenced.

RACE PCR Product exact sequence (243 bp): 5'- GGA CAC TGA CAT GGA CTG AAG GAG TAG AAA GAG CTC ATC AGA ACA GTC AGA CTG ATC AAG CTT CTC TAT CAA AGC AAC CCA CCT CCC AAT CCC GAG GGG ACG CGT CAG GCG CGC AGG AAT AGA AGG CGC CGG TGG AGA GAG AGA CAG AGA CAG ATC CAT TCG ATA TCT GAA CGG ATC CTT GGC ACT TAT CTG GGA CGA TCT GCA GAG CCT GTG CCT CTT CAG CTA CCA CCG CTT GAG AGG TTA -3'

For 21 mer duplex RNA, RACE PCR product was gel purified and directly sequenced using relative forward primer (5'-cDNA primer 1) and reverse primer (GSP primer 2).

RACE PCR Product exact sequence (249 bp):

5'- GGA CAC TGA CAT GGA CTG AAG GAG TAG AAA GAC GAA GAG CTC ATC AGA ACA GTC AGA CTG ATC AAG CTT CTC TAT CAA AGC AAC CCA CCT CCC AAT CCC GAG GGG ACG CGT CAG GCG CGC AGG AAT AGA AGG CGC CGG TGG AGA GAG AGA CAG AGA CAG ATC CAT TCG ATA TCT GAA CGG ATC CTT GGC ACT TAT CTG GGA CGA TCT GCA GAG CCT GTG CCT CTT CAG CTA CCA CCG CTT GAG AGG TTA -3'

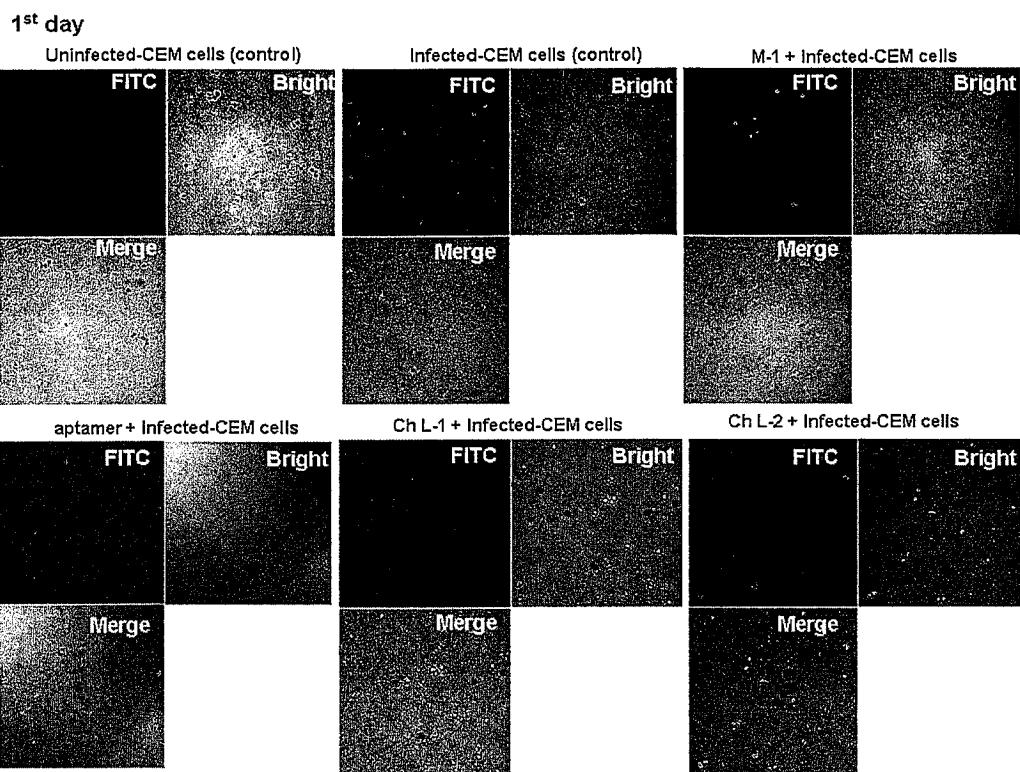
Target Site I

TGACCTCCATAGAACGACACCGGGACCGATCCAGCCCTCGCGGGCGCGCAAGAAATGGCTAGCGCAGGAAGAGCGGAGACAGC
GACGAAGAGCTCATCAGAACAGTCAGACTGATCAAGCTCTCTATCAAAGCAACCCACCTCCAATCCGAGGGGACCGCTAG
GCGCGCAGGAATAGAAGGGCGCCGGTGGAGAGAGAGACAGAGACAGATCCATTGATATCTGAACGGATCCTGGCACTTATCTGGGA
CGATCTGCAGAGCCTGTGCCTCTCAGCTACCACCGCTTGAGAGGTTAACTCTTGATTGTAACGAGGATTGGAACAGGGACACA
GGGTGTGGGGTCACTCAAATATTGGTGGAAATCTCTACAGTGGAGTCAGGAACATAAGAGAATGGTGCAAGGAGCTAGCAAAGGA
GAAGAACTCTCACTGGAGTTGTCCTAAATTCTGTTGAATTAGATGGTGTGTTAACGGCCACAAAGTTCTCTGTCAGTGAGAGGGTGA
GSP primer 1

27 mer duplex RNA:
RACE PCR 5'-adaptor product: 243 bp

21 mer duplex RNA:
RACE PCR 5'-adaptor product: 249 bp

Figure S4: a)



b)

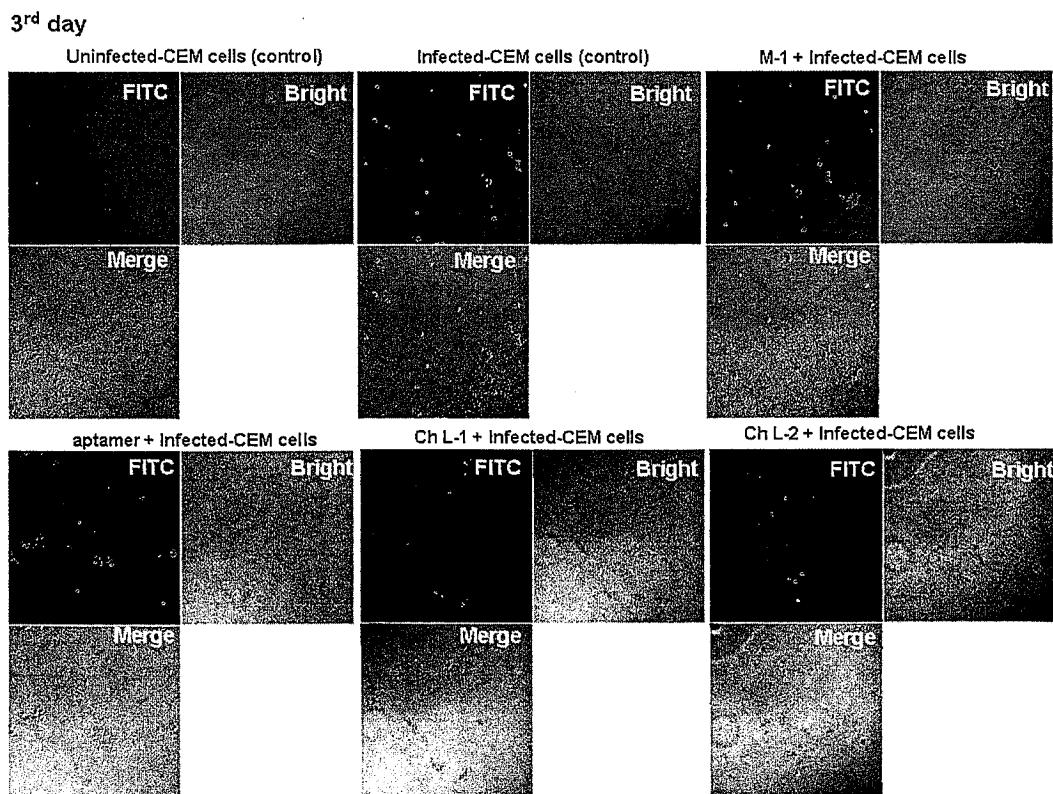


Figure S5:

